GENETIC AND KARYOTYPIC IDENTIFICATION OF *SACCHAROMYCES* YEASTS FROM FAR EAST ASIA

G.I. NAUMOV[1*], E.S. NAUMOVA[1], Z.M. AZBUKINA[2], M. KORHOLA[3] and C. GAILLARDIN[4]

1. State Institute for Genetics and Selection of Industrial Microorganisms, 1 Dorozhnyi 1, Moscow 113545, Russia.
2. Institute of Biology and Pedology, Vladivostok 690022, Russia.
3. Research Laboratories of The Finnish State Alcohol Company (ALKO Ltd.), POB 350, SF-00101 Helsinki, Finland.
4. Laboratoire de Génétique des Microorganismes INRA-CNRS, Centre de Biotechnologies Agro-Industrielles, Institut National Agronomique, F-78850 Thiverval-Grignon, France

ABSTRACT - Wild isolates of the biological species *Saccharomyces paradoxus* Batschinskaia was found for the first time in Far East Asia (Russian continental region) and identified by genetical and molecular criteria. Strains of both species *S. cerevisiae* Hansen and *S. kluyveri* Phaff et al. were also isolated.

RÉSUMÉ - Des isolats sauvages de l'espèce biologique *Saccharomyces paradoxus* Batschinskaia ont été trouvés pour la première fois en Asie Extrême Orientale (région continentale Russe) et identifiés par des critères génétiques et moléculaires. Des souches de *S. cerevisiae* Hansen et *S. kluyveri* Phaff et al. ont également été isolées.

KEY WORDS : hybridization analysis, electrophoretic karyotyping, *Saccharomyces paradoxus*, *S. cerevisiae*, *S. kluyveri*, taxonomy.

INTRODUCTION

According to genetic hybridization analysis and total DNA-DNA reassociation data, there are three biological sibling species within the taxonomical *Saccharomyces cerevisiae* yeast complex: *S. bayanus* Saccardo, *S. cerevisiae* Hansen, *S. paradoxus* Batschinskaya, and one hybrid taxon *S. pastorianus* Hansen (syn. *S. carlsbergensis* Hensen) (Naumov et al., 1983a; Naumov, 1987; Naumov & Nikonenko, 1989; Vaughan Martini, 1989; Vaughan Martini & Kurtzman, 1985; Vaughan Martini & Martini, 1987). The three sibling species are distinguished genetically, they can be crossed but interspecies hybrids remain sterile or yield non-viable ascospores. The *Saccharomyces cerevisiae* complex, formerly known as *Saccharomyces sensu stricto* (van der Walt, 1970), is not closely related to another group, *Saccharomyces sensu lato*, which in-

* Correspondence to Dr. C. Gaillardin.

cludes *S. kluyveri* Phaff et al., *S. dairensis* Naganishi, *S. exiguus* Hansen, *S. servazzii* Capriotti and *S. unisporus* Jörgensen (Johnston & Mortimer, 1986; Kaneto et al., 1989; Mikata, 1989; Vaughan Martini & Kurtzman, 1988; Yarrow, 1984).

During many years, *Saccharomyces sensu stricto* yeasts from East and West Europe have been intensively studied in order to find wild strains of the domesticated species *S. cerevisiae* (Naumov, 1980, 1986, 1987, 1988, 1989; Naumov & Nikonenko, 1987, 1988a; Naumov et al., 1992a). From natural substrates, away from human activities, such as oak exudates, uncultivated soils or insects, only the biological species *S. paradoxus* is found constantly. The second sibling species *S. bayanus* able to ferment melibiose occurs in wine prepared at low temperature (Naumov, 1987; Naumov & Nokonenko, 1989; Naumova et al., 1991). In previous studies we described two Asian populations of wild *S. cerevisiae* yeasts located far from one another: one in Central Siberia (Novosibirsk) and another in Japan (Naumov & Nikonenko, 1988b, Naumov & Naumova, 1991). It was thus proposed that European cultivated *S. cerevisiae* yeasts, which are now wide-spread over the world, originated from Far East Asia.

In the present study we identified *Saccharomyces* strains isolated in Russian Far East from regions located not far from Japan. Such floristic study will permit better evaluation of endemicity of *Saccharomyces* yeasts from Japan and, on the other hand, elucidation of the origin of European cultural yeasts.

MATERIALS AND METHODS

Strains

The list of *Saccharomyces* strains studied and their origin are presented in Table 1. The methods for cultivation, sporulation and hybridization of yeasts have been described elsewhere (Naumov et al., 1986). The sporulated cultures were treated with stomach juice of garden snails Helix to destroy ascus walls and then ascospores were isolated with a micromanupulator (Johnston & Mortimer, 1959). The method used to isolate natural strains was described before (Naumov et al., 1992a). Yeasts were collected from exudates of *Quercus mongolica* during expeditions in September-October 1987. Twelve pure cultures of *Saccharomyces* were isolated from mixed populations of yeasts, filamentous fungi and bacteria. Preliminary identification of the *Saccharomyces* yeasts was done according to size and morphology of ascospores (Yarrow, 1984).

Highly fertile monosporic cultures of *S. cerevisiae* VKM Y-502 (CBS 5287), SBY 2576 and *S. paradoxus* CBS 5829 strains marked by auxotrophic ade mutations (red colonies) were used as genetic species testers (Naumov, 1987; Naumov et al., 1983b). Chromosomal DNAs prepared from strains of *S. cerevisiae* (YNN 295), *S. paradoxus* (CBS 432) and *S. kluyveri* (NRRL 12651, CBS 4570) were used as standards for karyotyping studies. The following acronyms of culture collections are used: VKM = National Collection of Microorganisms, Moscow, Russia; SBY = Seccion de Bioquimica, Instituto National de Investigaciones Agrarias, Madrid, Spain; CBS = Centraalbureau voor Schimmelcultures, Delft, The Netherlands; NRRL = Northern Region Research Center, Peoria, Ill., U.S.A.

CHEF gel electrophoresis

Preparation of chromosomal DNAs have been described before (Naumov et al., 1991). A CHEF-DR™ II apparatus (Bio-Rad, Richmond, CA, U.S.A.) was used to separate the chromosomal DNAs.The electrophoresis buffer was 0.5 x TBE. The buffer was circulated around the gel and cooled to 14 C. Electrophoresis was carried out at

Table 1 - List of the *Saccharomyces* strains studied and isolated from exudates of *Quercus mongolica* in Far East of Russia.

Tableau 1 - Liste des souches de *Saccharomyces* isolées d'exsudats de *Quercus mongolica* en Extrême Orient Russe.

Strain designation	Place of isolation	Date of isolation
N°42	Botanical garden in the environs of Vladivostok	06.VIII.87
N°43	The cape "Peschannyi" in the environs of Vladivostok	12.VIII.87
N°44	The environs of Ternei city	16.VIII.87
N°45	The environs of Ternei city	16.VIII.87
N°46	"Blagodatnoe" department of the nature reserve "Sikhote-Alinsky"	17.VIII.87
N°47	"Blagodatnoe" department of the nature reserve "Sikhote-Alinsky"	17.VIII.87
N°48	Biological station of Far East State University, v. Rjazanovka of Khazansky district	28.VIII.87
N°49[1]	Biological station of Far East State University, v. Rjazanovka of Khazansky district	28.VIII.87
N°50	The railway station "Chajka", Vladivostok	02.IX.87
N°51	The railway station "Chajka", Vladivostok	02.IX.87
N°52	The nature reserve "Kedrovaya pad"	09.VIII.87
N°53	"Blagodatnoe" department of the nature reserve "Sikhote-Alinsky"	17.VIII.87

[1]The strain was isolated from exudate of *Q. dentata*.

200 V for 15h with ■ switching time of 60 s and then for 8h with ■ switching time of 90s. For better separation of chromosomal DNAs of *S. kluyveri* strains electrophoresis

was carried out at 200 V for 15 h with a switching time of 70s and then for 8 h with a switching time of 120 s.

RESULTS AND DISCUSSION

Monosporic cloning

For genetic analysis, monosporous, highly fertile strains should be used. Such strains with homozygous chromosome sets are also more suitable for electrophoretic karyotyping because they do not carry chromosomes. To obtain highly fertile lines 6-10 asci (tetrads) were dissected in each strain. All strains studied were homothallic with very high ascospore viability in the range of 85-100%.

Two strains N° 52 and N° 53 were not suitable for genetic analysis because their ascus walls were not destroyed by the enzyme treatment. In this respect they are similar to strains of *Saccharomyces kluyveri* (McCullough & Herskowitz, 1979).

Electrophoretic karyotyping

All strains under examination were compared by electrokaryotyping (Fig. 1A). Karyotyping patterns of 10 strains (N° 42-51) were very similar to each other and to those of standard testers of *S. cerevisiae* and *S. paradoxus*. These two species are known to display similar karyotyping patterns, while karyotypes of *S. bayanus* were clearly distinguishable from those of *S. cerevisiae* and *S. paradoxus* (Naumova et al., 1991; Naumov et al., 1992b). So, it was possible to conclude that the strains studied belonged either to *S. cerevisiae* or to *S. paradoxus*, but not to *S. bayanus*. Karyotyping pattern of strain N° 43 (Fig. 1A, lane 5) was slightly different from the others for chromosomes VII and XV, which usually migrate together, and for chromosomes V, VIII and IX which differ in size from the other strains.

The two strains N° 52 and N° 53 were very different in terms of karyotype from other *Saccharomyces* strains studied (Fig. 1A, lane 14, 15). Chromosomal DNA of these strains was resolved into 6 bands with chromosome sizes ranging from 2.2 Mb to 1.0 Mb, while other strains including standards showed 12-15 bands ranging from 2.2 Mb to 245 Kb. Karyotyping profiles of these two strains corresponded more closely to that of *S. kluyveri* (Johnston et al., 1988). Karyotyping patterns of strains N° 52 and N° 53 were further compared with two strains of *S. kluyveri* including the type culture NRRL Y-12651 (Fig. B). All four strains showed identical karyotypes, suggesting that strains N° 52 and N° 53 may belong to the species *S. kluyveri*. This suggests that it may now be possible to identify by molecular karyotyping the species *S. kluyveri* (data of the present study), *S. unisporus* (Mikata, 1989), *S. bayanus* and the complex *S. cerevisiae* - *S. paradoxus* (Noumova et al., 1991; Naumov et al., 1992b.

Genetic hybridization analysis

Monosporic cultures of 10 strains (N° 42-51) were crossed with *S. cerevisiae* VKM Y-502 and *S. paradoxus* CBS 5829 tester strains. Crosses were made by the "spore-to-spore" mating method using a micromanipulator. Zygote formation was observed in all crosses. These experiments confirms the karyotyping data and show that all strains 42 to 51 belong to the complex *Saccharomyces cerevisiae*. To establish the fine species belonging of the strains, all hybrids were studied by tetrad analysis (Table 2, 3). Species assignment of the strains was determined on the basis of the viability of hybrid ascospores. Viability of hybrid ascospores and regular meiotic segregation of control *ade* marker indicated the high homology of the genomes of the parent strains

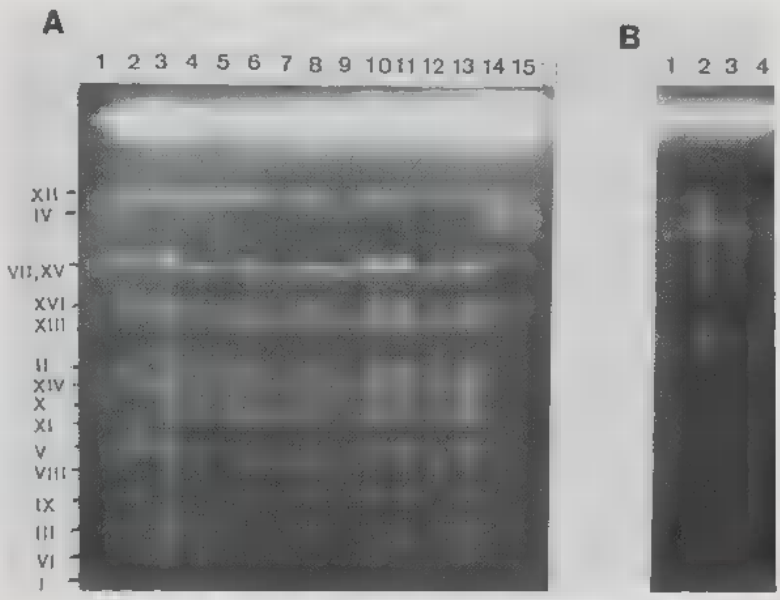

Figure 1 - CHEF gel electrophoresis analysis of chromosomal DNAs from natural *Saccharomyces* strains isolated in Russian Far East and from standard strains of *S. cerevisiae*, *S. paradoxus* and *S. kluyveri*.
(A) Lanes 1, 2 and 13, *S. cerevisiae*, YNN 295, X2180-1A and N 51, respectively; lanes 3-12, *S. paradoxus*, CBS 432, N 42, N 43, N 44, N 45, N 46, N 47, N 48, N 49 and N 50, respectively; lanes 14 and 15, *S. kluyveri*, N 52 and N 53, respectively.
(B) Lanes 1-4, *S. kluyveri*, NRRL Y-12651, CBS 4570, N 52 and N 53, respectively.

All cultures studied are monosporic isolates, with the exception of *S. kluyveri* ones.

Figure 1 - Analyse électrophorétique en gel CHEF d'ADN chromosomique d'isolats naturels de *Saccharomyces* d'Extrême Orient Russe et de souches de références de *S. cerevisiae*, *S. paradoxus* et *S. kluyveri*.
(A) Pistes 1, 2 et 13, *S. cerevisiae*, YNN 295, X2180-1A et N 51; pistes 3-12, *S. paradoxus*, CBS 432, N 42, N 43, N 44, N 45, N 46, N 47, N 48, N 49 et N 50; pistes 14 et 15, *S. kluyveri*, N 52 et N 53.
(B) Pistes 1-4, *S. kluyveri*, NRRL Y-12651, CBS 4570, N 52 et N 53.

Toutes les cultures étudiées proviennent d'isolements monosporaux, à l'exception de *S. kluyveri*.

and their belonging to the same biological species (Table 2). Conversely, non viability of hybrid ascospores enabled us to assign the corresponding parents to different species (Table 3). According to the results obtained, strains 42 to 50 belong to the species *S. paradoxus*. Strain N° 51 corresponds to the species *S. cerevisiae* sensu stricto; intra-species control crossing of *S. cerevisiae* 502 x 2576 gave normal meiotic segragation (Table 2).

Thus, three species *S. paradoxus* (9 strains), *S. cerevisiae* (1 strain) and *S. kluyveri* (2 strains) were found among wild *Saccharomyces* yeasts of the continental part of Russian Far East. *S. cerevisiae* strain N° 51 was isolated in Vladivostok not far from dwelling buildings. Rare semicultivated wine yeasts of *S. cerevisiae* have been formerly identified in Far East of Russia (Naumov, 1988, 1990a, b). In the continental

Table 2 - Genetic analysis of intraspecies hybrids of *S. paradoxus* and *S. cerevisiae*.

Tableau 2 - Analyse génétique d'hybrides intraspécifique de *S. paradoxus* et *S. cerevisiae*.

Origin of hybrids	No. of spore pairs crossed	No. of zygotes obtained	No. of tetrads isolated	Proportion of viable ascospores of hybrids (%)	Segregation of *ade*:*ADE*[1]
		S. paradoxus x *S. paradoxus*			
42 x 5829	32	6	38	45	35:33
43 x 5829	29	6	45	24	15:29
44 x 5829	30	2	44	55	41:44
45 x 5829	33	2	34	33	22:23
46 x 5829	35	9	45	39	33:38
47 x 5829	37	7	44	43	37:38
48 x 5829	30	7	36	45	31:34
49 x 5829	32	5	40	46	40:33
50 x 5829	31	5	41	50	41:41
		S. cerevisiae x *S. cerevisiae*			
51 x 502	'34	2	12	92	2:2 (10)
502 x 2576	46	5	25	86	2:2 (16)

[1]Data of random spore or tetrad analysis are presented. Number of tetrads is indicated in parenthesis.

part of Russian Far East, wild yeast of the complex *S. cerevisiae* correspond more likely to the species *S. paradoxus* than to the species *S. cerevisiae* sensu stricto. On the contrary in Japan, *S. cerevisiae* strains are common in nature (Yoneyama, 1957, 1958; Kodama, 1974; Banno, 1975; Banno & Mikata, 1981; Naumov & Nikonenko, 1988b). It thus seems that Japanese populations of wild *S. cerevisiae* yeasts are unique for the Far East Asia.

It should be noted that North American and Far East Asian strains of *S. kluyveri* are more homogenous in terms of karyotypes (Fig. 1B) than strains of South Africa (Coetzee et al., 1987).

In this and previous investigations (Naumov & Nikonenko, 1988b) we obtained preliminary data on the distribution of *Saccharomyces cerevisiae* and *S. paradoxus* yeasts in Far East Asia. More studies of *Saccharomyces* yeasts of Sakhalin and Kuril Islands, the enlarged screenings of strains of the continental Far East region and Japan, have to be done before definite conclusions can be drawn regarding the species composition of wild populations of *Saccharomyces* in Far East Asia.

ACKNOWLEDGEMENTS

This research was supported by Far Eats Department of Russian Academy of Sciences, Research Laboratories of the Finnish State Alcohol Company (ALKO Ltd.), by grants of Institut National de la Recherche Agronomique and of Ministère de la Recherche et de la Technologie (France).

Table 3 - Genetic analysis of interspecies hybrids *S. paradoxus x S. cerevisiae.*

Tableau 3 - Analyse d'hybrides interspécifiques entre *S. paradoxus* et *S. cerevisiae.*

Origin of hybrids	No. of spore pairs crossed	No. of zygotes obtained	No. of tetrads isolated	Proportion of viable ascospores of hybrids (%)
42 x 502	29	2	26	0[1]
43 x 502	26	[illegible]	27	[illegible]
44 x 502	29	3	35	0
45 x 502	27	4	37	0
46 x 502	31	2	41	0
47 x 502	36	4	26	0
48 x 502	23	4	39	0
49 x 502	23	2	41	0
50 x 502	30	4	31	0
5829 x 51	75	6	44	0

[1]Hybrids of strains NN 42, 44, 45, 46, 47 and 49 with the *S. cerevisiae* tester formed 1-2 microcolonies.

REFERENCES

BANNO I., 1975 - *Saccharomyces* yeasts isolated in Japan: (1) A numerical analysis of *S. cerevisiae* and its allied species. *IFO Res. Comm.* 7: 15-23.

BANNO I. and MIKATA K., 1981 - Ascomycetous yeasts isolated from forest materials in Japan. *IFO Res. Comm.* 10: 10-19.

COETZEE D.J., KOCK J.L.F. and PRETORIUS G.H.J., 1987 - The value of orthogonal-field-alternation gel electrophoresis as [illegible] rapid identification process for some species representing the genus *Saccharomyces. J. Microbiol. Meth.* 7: 219-225.

JOHNSTON J.R., CONTOPOULOU C.R. and MORTIMER R.K., 1988 - Karyotyping of yeast strains of yeast strains of several genera by field inversion gel electrophoresis. *Yeast* 4: 191-198.

JOHNSTON J.R. and MORTIMER R.K., 1959 - Use of snail digestive juice in isolation of yeast spore tetrads. *J. Bacteriol.* 78: 292.

JOHNSTON J.R. and MORTIMER R.K., 1986 - Electrophoretic karyotyping of laboratory and commercial strains of *Saccharomyces* and other yeasts. *Int. J. System. Bacteriol.* 36: 569-572.

KANEKO Y., MIKATA K. and BANNO I., 1989 - Karyotyping of *Saccharomyces exiguus* by pulsed-field gel electrophoresis. *IFO Res. Comm.* 14: 111-117.

KODAMA K., 1974 - Ascosporogenous yeasts isolated from tree exsudates in Japan. *Ann. Microbiol. Enzimol.* 24: 215-231.

McCULLOUGH J. and HERSKOWITZ I., 1979 - Mating pheromone interactions between *Saccharomyces kluyveri* and *Saccharomyces cerevisiae. J. Bacteriol.* 138: 146-154.

MIKATA K., 1989 - Descriptive catalogue of IFO yeast. Collection VI. *IFO Res. Comm.* 14: 153-155.

NAUMOV G.I., 1980 - The biological species *Saccharomyces terrestris*. *Dokl. Biol. Sci.* 249: 1248-1250.

NAUMOV G.I., 1986 - Genetic differentiation and ecology of the yeast *Saccharomyces paradoxus* Batschinskaia. *Dokl. Biol. Sci.* 289-291: 213-216.

NAUMOV G.I., 1987 - Genetic basis for classification and identification of the ascomycetous yeasts. *Stud. Mycol.* 30: 469-475.

NAUMOV G.I., 1988 - A hybridological study of the yeast *Saccharomyces* from the expedition collection of V.I. Kudriavzev (during 1934 and 1936). *Mikol. Fitopatol.* 22: 296-301 (in Russian)

NAUMOV G.I., 1989 - Occurrence of *Saccharomyces paradoxus* in Estonia. Eesti NSV Teaduste Akademia Toimetised. *Biologia* 39: 9-12 (in Russian).

NAUMOV G.I., 1990a - A study of yeasts from Far East. *In:* L.N. VASILIEVA, Z.M. AZBUKINA and L.H. EGOROVA, *Cryptogamic Studies in Far East.* Vladivostok, USSR Academy of Sciences, 72-75 (in Russian).

NAUMOV G.I., 1990b - Genetic polymorphism of *Saccharomyces cerevisiae* strains from Far East. *In:* L.N. VASILIEVA Z.M. AZBUKINA and L.H. EGOROVA, *Cryptogamic studies in Far East.* Vladivostok, USSR Academy of Sciences, 76-80 (in Russian).

NAUMOV G.I., KONDRATIEVA V.I. and NAUMOVA E.S., 1986 - Methods for hybridization of homothallic yeast diplonts and haplonts. *Soviet Biotechnol.* 6: 29-32.

NAUMOV G.I., KONDRATIEVA V.I., NAUMOVA T.I. and GUDKOVA N.K., 1983a - Genetic basis for classification of *Saccharomyces cerevisiae*. A study of survival of hybrid ascospores. *Zhurn. Obshchei Biol.* 44: 648-660 (in Russian).

NAUMOV G.I., KONDRATIEVA V.I., NAUMOVA T.I. and GUDKOVA N.K., 1983b - Genetic inbred lines of yeasts *Saccharomyces cerevisiae* Hansen. *Mikol. Fitopatol.* 17: 476-480 (in Russian).

NAUMOV G.I. and NAUMOVA E.S., 1991 - A wild yeast population of *Saccharomyces cerevisiae* found in Siberia. *Microbiologia* 60: 137-140 (in Russian).

NAUMOV G., NAUMOVA E. and KORHOLA M., 1992a - Genetic identification of natural *Saccharomyces* sensu stricto yeasts from Finland, Holland and Slovakia. *Antonie van Leeuwenhoek J. Microbiol. Sevol.* 61: 237-243.

NAUMOV G.I., NAUMOVA E.S., LANTTO R.A., LOUIS E.J. and KORHOLA M., 1992b - Genetic homology between *Saccharomyces cerevisiae* and its sibling species *S. paradoxus* and *S. bayanus*: electrophorectic karyotypes. *Yeast* 8: 599-612.

NAUMOV G., NAUMOVA E., TURAKAINEN H., SUOMINEN P. and KORHOLA M., 1991 - Polymeric genes MEL8, MEL9 and MEL10 - new members of -galactosidase gene family in *Saccharomyces cerevisiae*. *Curr. Genet.* 20: 269-276.

NAUMOV G.I. and NIKONENKO T.A., 1987 - Genomic divergence in cultivated and wild strains of the *Saccharomyces* sensu stricto: four twin species. *Dokl. Biol. Sci.* 294: 330-332.

NAUMOV G.I. and NIKONENKO T.A., 1988a - New isolate of *Saccharomyces paradoxus* yeasts from oak exsudates. *Biol. Nauki (Alma-Ata)* 7: 84-87 (in Russian).

NAUMOV G.I. and NIKONENKO T.A., 1988b - The East Asia is a probable land of the cultures yeasts *Saccharomyces cerevisiae*. *Izv. Sibirsk. Otd. Akad. Nauk S.S.S.R., Ser. Biol. Nauk* 20: 97-101 (in Russian).

NAUMOV G.I. and NIKONENKO T.A., 1989 - Occurrence and physiological characteristics of biological species *Saccharomyces bayanus* from hybridological analysis. *Microbiology* 57: 526-530.

NAUMOVA E.S., NAUMOV G.I., MICHELS C.A. and BERITASHVILI D.R., 1991 - Identification of chromosomal DNA patterns of the species *Saccharomyces bayanus* and *S. pastorianus*. *Dokl. Akad. Nauk. S.S.S.R.* 316: 744-746 (in Russian).

van der WALT J.P., 1970 - Genus 16. *Saccharomyces* Meyen emend. Reess. In: J. Lodder, *The Yeasts, a taxonomic study*. Amsterdam, North-Holland Publishing Company, 555-718.

VAUGHAN MARTINI A., 1989 - *Saccharomyces paradoxus* comb. nov., a newly separated species of the *Saccharomyces* sensu stricto complex based upon nDNA/homologies. *Syst. Appl. Microbiol.* 12: 179-182.

VAUGHAN MARTINI A. and KURTZMAN C.P., 1985 - Deoxyribonucleic acid relatedness among species of the genus *Saccharomyces* sensu stricto. *Int. Syst. Bacteriol.* 35: 508-511.

VAUGHAN MARTINI A. and KURTZMAN C.P., 1988 - Deoxyribonucleic acid relatedness among species of *Saccharomyces* sensu lato. *Mycologia* 80: 241-243.

VAUGHAN MARTINI A. and MARTINI A., 1987 - Three newly delimited species of *Saccharomyces* sensu stricto. *Antonie van Leuwenhoek. J. Microbiol. Serol.* 53: 77-84.

YARROW D., 1984 - Genus 22. *Saccharomyces* Meyen ex Reess. *In:* N.J.W. KREGER-VAN RIJ, The yeasts, a taxonomic study. Amsterdam, Elsevier Sci. Publ., 379-395.

YONEYAMA M., 1957 - Studies on natural habitats of yeasts bark- inhabiting yeasts. *J. Sci. Hiroshima Univ.*, Ser. B., Div. 2, 8: 19-38.

YONEYAMA M., 1958 - Studies on natural habitats of yeasts, wild yeasts closely related to *Saccharomyces cerevisiae* Hansen. *J. Sci. Hiroshima Univ.*, Ser. B, Div. 2, 8: 145-165.